protein yielded similar results. However, sedimentation equilibrium analysis yielded dissociation constants of 160 to 280 nM. Temperature and pressure variations did not explain this difference. Small amounts of degradation over the several day sedimentation equilibrium protocol was revealed by silver-stained SDS-PAGE and likely underlies the different Kds for sedimentation velocity and sedimentation equilibrium experiments. Our results confirm that the GluA2 ATD forms nM affinity dimers3. The spread of values measured by SV highlights the difficulty in making accurate measurements at nanomolar protein concentrations, due to dimer-deficient monomers produced by proteolysis or extremely small signal to noise ratios. The similar variance observed in prior work with fluorescence detection AUC suggests that S/N limitations are not the major cause of Kd variations.

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1705-Pos Board B475

A Novel Toxin that Targets Acid-Sensing Ion Channels to Produce Pain

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Natural products have served as invaluable keys to unlocking molecular underpinnings of pain sensation. In particular, plant-derived irritants, such as capsaicin, menthol, mustard oil, have proven essential for identifying and/or characterizing excitatory TRP ion channels that play major roles in pain sensation. Animals also possess chemical defense mechanisms to inflict pain in predators. To more fully exploit the rich pharmacopeia of animal toxins, we established an unbiased screen to discover venoms capable of activating specific subpopulations of somatosensory neurons, with the goal of developing novel probes and identifying important physiological targets for pain research and therapeutics. We show that venom from the Texas coral snake - whose bite produces excruciating pain - contains a novel toxin that potently and persistently activates a subset of somatosensory neurons. First, we use biochemical and molecular methods to identify a unique two-component toxin that form a high affinity heteromeric complex. Second, we use electrophysiological and genetic approaches to identify acid-sensing ion channels (ASICs) as the molecular target. Furthermore, behavioral experiments show that toxin-evoked activation of ASIC channels elicits pain by recruiting a canonical population of primary afferent nociceptors that detect thermal and inflammatory pain.

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Conformational Change Involved in Gating of Acid Sensing Ion Channel (ASIC1a)

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Acid sensing ion channels are proton activated, sodium selective channels and are trimeric in nature. They belong to the epithelial sodium channel family. They are widely expressed in peripheral sensory neurons and neurons of central nervous system. These channels are involved in various physiological functions like sodium homeostasis, pain, mechano sensation, acidosis induced neuronal injury, etc. Here we expressed a modified chicken acid sensing ion channel (ASIC1a) in insect cells containing engineered cysteine residues at positions 129 and 338, at the finger and thumb domain respectively. The membrane preparations of the protein expressed in insect cells was shown to be functional using bilayer measurements. Using this functional construct we performed Fluorescence Resonance Energy Transfer measurements by tagging the insect cells with terbium chelate as donor and ATTO 465 as the acceptor. The distance between the donor and acceptor was similar to that observed in crystal structure thus indicating that the crystal structure is a good representation of the functional receptor in the membranes. Additionally, decreasing the pH from 7.4 to 6 resulted in a decrease in distance between the two residues, consistent with the movement of the thumb domain closer to the finger domain as previously hypothesized based on the crystal structure.

1707-Pos Board B477

Modulation of Human Acid-Sensing Ion Channel 1A Open Channel Inactivation by FRRFamide

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The ASICs (Acid-Sensing Ion Channels) are involved in neuronal signaling in the central and peripheral nervous system. These non-voltage-gated channels are involved in learning, the expression of fear, neurodegeneration after ischemia, and pain sensation. The molecular bases underlying their activity are not yet fully understood. During an extracellular acidification, ASICs open transiently before inactivating in the continued presence of the low extracellular pH. Modulators of ASIC inactivation may contribute to the physiological and pathological functions of ASICs. FRRFamide (FRRFa) and related peptides have been shown to slow the ASIC inactivation time course and to induce a small sustained current. In the present study we have carried out in silico docking of FRRFa to human ASIC1a, which predicted two cavities as FRRFa binding site. It has previously been shown that a part of the thumb region differs between ASIC1 orthologs, and that FRRFa induces greater sustained currents in human than in mouse ASIC1a, suggesting that this region may be involved in the effect of FRRFa.

The role of the residues predicted to be part of the FRRFa binding site (one of the top docking poses) has been tested by site-directed mutagenesis and functional studies. While mutation of only one residue of the predicted binding site decreased the effect of FRRFa, several point mutations in the β9-α4 region increased the FRRFa-induced sustained current. Our results indicate that the β9-α4 region is likely not the FRRFa binding site, however that it is involved in the effect of FRRFa on ASIC inactivation.

1708-Pos Board B478

AFM Imaging Reveals the Assembly of a P2X Receptor Complex Containing P2X2, P2X4 and P2X6 Subunits

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Seven P2X purinergic receptor subunits have been identified: P2X1-P2X7. All except P2X6 assemble as homotrimers, and six heteromeric receptors (P2X1/2, P2X1/4, P2X1/5, P2X2/3, P2X2/6 and P2X4/6) have been described. In addition, P2X4 homomers associate with P2X2 or P2X7 homomers as dimers of trimers. The various P2X receptors show individual functional properties, suggesting distinct physiological roles. The overlapping expression of P2X2, P2X4 and P2X6 subunits has been shown in different cell types, and functional analysis of P2X receptors in Leydig cells suggests that the three subunits interact. In the present study we investigated the potential assembly of P2X2, P2X4 and P2X6 subunits into heteromeric receptors. tsA 201 cells were co-transfected with His6-tagged P2X2, HA-tagged P2X4 and FLAG-tagged P2X6 subunits. After sequential co-immunoprecipitation using anti-HA and anti-FLAG resins, all three subunits were present, demonstrating their interaction. Proteins eluted from the resins were incubated with anti-His6 antibodies and anti-HA Fab fragments, and analyzed by atomic force microscopy (AFM). In 292 AFM images, 23 central particles with volumes expected for P2X trimers were found to be doubly decorated by one antibody and one Fab fragment. In contrast, only two such complexes were seen when the antibody/Fab incubation was omitted (223 images). Two complexes were also seen after incubation with anti-Myc antibodies plus anti-V5 Fab fragments (control; 263 images). This result is consistent with the presence of a P2X2/4/6 heterotrimer.

We conclude that P2X2, P2X4 and P2X6 subunits interact, potentially forming a heterotrimeric receptor containing three different subunits.

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Subtype Specific Activation of P2X Receptors by Free and Magnesium-Bound ATP

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P2X receptors are cation-selective channels that were suggested to play roles in many important physiological processes, including muscle contraction, pain sensation, and inflammation. Extracellular ATP released from various sources, such as synaptic vesicles and damaged cells, is the ligand for activating P2X receptors. In neutral solution, ATP is ionized and exists mostly as free ATP (ATP^{4-}), a high affinity chelator for divalent and trivalent cations. As there

are millimolar divalent cations present in the extracellular milieu, it is likely that most extracellular ATP released from synaptic vesicles is chelated by divalent. We found that some subtypes of P2X receptors can be activated by both free and divalent-bound ATP, while others can only be efficiently activated by free ATP. This subtype specific activation by different forms of ATP parallels the pharmacological sensitivity to other agonists and antagonists, pointing to the existence of two distinct classes of ligand binding pockets. We are currently examining which forms of ATP activate heteromeric P2X receptor channels formed by subunits with different sensitivity to divalent-bound ATP.

1710-Pos Board B480

Functional P2X7 Receptor Expression in the Magnocellular Neurons of the Hypothalamic Neurohypophysial System (HNS)

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Our laboratory has presented evidence for P2x7 receptors (P2x7R) in isolated terminals of the neurohypophysis (NH) (Cuadra et al., 2010, *Biophys. J.***97***:* 1474). In this study we further examined P2x7R immunoreactivity (IR) and function. A stereo-specific ecto-P2x7R antibody was used for IR studies (Kim el al., 2001, JBC, **276**:23262) in the rat HNS: supraoptic nucleus (SON), paraventricular nucleus (PVN) and NH. We found limited P2x7R IR in the SON and the PVN, which was absent from the somata of OT- and AVP-neurons. P2x7R IR was co-localized in some cell bodies containing the glial specific marker, GFAP. In contrast, P2x7R IR was abundantly seen on the membrane of NH terminals (NHT), with dense IR in terminals lining the capillary borders. This was confirmed in isolated NHT that showed IR puncta on the membranes of both AVP- and OT-containing terminals. Initial results using ratiometric calcium imaging with Fura-2 in isolated NHT showed differential $[Ca^{2+}]_i$responses to 100 μM *vs*. 1 mM ATP; some NHT responded to both doses while others responded only to 1 mM ATP. A similar distribution in response was observed with ATP generated ion currents during patch clamp recordings. NHT responded with either high sensitivity (HS) or low sensitivity (LS) to ATP. The HS group corresponded to AVP-NHT, which is reported to express P2x2R, P2x3R, P2x4R and P2x7R (Knott et al., 2005, Pfluger Arch, **405**:381). In contrast, the LS group corresponded to OT-NHT, which is consistent with a P2x7R response. Together these data suggest that in the HNS P2x7R is expressed chiefly in the AVP- and OT-secreting terminals of magnocellular neurons. (Supported by NIH grant NS29470 to JRL)

1711-Pos Board B481

Understanding the Kinetics of ATP-Activated P2X2A and P2X2B Receptor Channels using a Markov State Model

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ATP-gated P2X2 receptors exhibit two opposite activation-dependent changes during sustained agonist application, pore dilation and pore closing (desensitization), through a process that is incompletely understood. To address this issue and to clarify the roles of Ca^{2+} and the C-terminal domain in gating, we combined biophysical and mathematical approaches using the full size receptor (labeled P2X2aR) and the splice form missing 69 residues in the C-terminal domain (labeled the P2X2bR). Both forms of the receptor developed conductivity for large organic cations within 2-6 s of ATP application and desensitized in a Ca^{2+} influx-dependent manner, whereas P2X2bR also desensitized in a Ca^{2+} influx-independent manner. In whole-cell recording with broken membranes, we also observed use-dependent facilitation of desensitization, reflecting the altered Ca^{2+} handling by cells. Such behavior was accounted for by a Markov state kinetic model with 12 states describing the ATP binding/unbinding and activation/desensitization. The model assumes that naïve receptors open when two ATP molecules bind and slowly dilates to a higher conductance state when a third ATP binds, generating a shift to less negative reversal potential observed experimentally in organic cation-containing medium. The use-dependent desensitization is modeled by a Ca^{2+}-dependent toggle switch, whereas the P2X2bR model also exhibits fast Ca^{2+}-independent desensitization. The model is extended to include memory to previous stimulations that not only explained the decrease in the slope of the IV-curves during −80 to +80 voltage ramps delivered twice per second, but also captured the effect of ATP stimulation when cells were held at positive holding potential.

1712-Pos Board B482

The Effect of Anions on the Human P2X7 Receptor

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P2X7 receptors (P2X7Rs) are nonselective cation channels that are opened by the binding of extracellular ATP and are involved in the modulation of epithelial secretion, inflammation and nociception. Here, we investigated the effect of extracellular anions on channel gating and permeation of human P2X7Rs (hP2X7Rs) expressed in *Xenopus laevis* oocytes. Two-microelectrode voltage-clamp recordings showed that ATP-induced hP2X7R-mediated currents increased when extracellular chloride was substituted by the organic anions glutamate or aspartate and decreased when chloride was replaced by the inorganic anions nitrate, sulfate or iodide. ATP concentration-response comparisons revealed that substitution of chloride by glutamate decreased agonist efficacy, while substitution by iodide increased agonist efficacy at high ATP concentrations. Meanwhile, the ATP potency remained unchanged. Activation of the hP2X7R at low ATP concentrations via the high-affinity ATP effector site was not affected by the replacement of chloride by glutamate or iodide. To analyze the anion effect on the hP2X7R at the single-molecule level, we performed single-channel current measurements using the patch-clamp technique in the outside-out configuration. Chloride substitution did not affect the single-channel conductance, but the probability that the P2X7R channel was open increased when chloride was replaced by glutamate and decreased when chloride was replaced by iodide. This effect was due to an influence of the anions on the mean closed times of the hP2X7R channel. We conclude that hP2X7R channels are not anion-permeable in physiological Na^+-based media and that external anions allosterically affect ion channel opening in the fully ATP^{4-}-liganded P2X7R through an extracellular anion binding site.

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Direct Permeation of the P2X7 Receptor Pore by Nanometer Molecules

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The selectivity of ion channels is generally considered to be fixed. The ATP-gated P2X7 receptor is unusual in that its activation leads to a progressive increase in the permeation of large cations during several seconds (*e.g.* YO-PRO-1). However, controversy remains whether these large molecules directly permeate the P2X7 receptor pore or use some coupled transport mechanism. In HEK293 cells expressing the rP2X7 receptor, simultaneous measurement of ionic current and YO-PRO-1 influx showed they both increased concomitantly with the application of ATP. At positive potentials YO-PRO-1 influx was much reduced, implying that movement of this large cation (1.8 x 0.8 x 0.7 nm) is influenced by the membrane electric field in a manner similar to smaller cations. Increasing the positivity in the pore of the P2X7 receptor significantly enhanced chloride permeability (D352N and T348K; P_{Cl}/P_{Na} increased 10-fold compared to WT). The selective permeability of large molecules was then measured using two ions of similar size and structure, bearing either a positive or a negative net charge: both the cation (ethidium: 1.2 x 1.0 x 0.5 nm) and the anion (FITC: 1.3 x 1.1 x 0.7 nm) were simultaneously detected by intracellular fluorescence. Introduction of a positive charge in the pore (T348K) or removal of a negative charge (D352N) increased the influx of the anion FITC, and decreased the influx of the cation ethidium. These effects parallel the findings of chloride permeability, demonstrating that by increasing the positivity in the pore of the P2X7 receptor the permeation pathway is more energetically favourable for anions, whether they are small or large. This implies that large molecules directly permeate the P2X7 receptor pore and any model of channel opening should accommodate a pore diameter >1 nm.

1714-Pos Board B484

The Gating Mechanism of a P2X4 Receptor: Normal Mode Analysis and Molecular Dynamics Simulations

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P2X4 receptors are trimeric ATP-gated non-selective cation channels which play crucial roles in various physiological processes. It remains unclear how ATP binding triggers channel opening. Here, we propose a gating mechanism